FORM PTO-1390 (REV. 1-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 20560-27

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/719945

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AT99/00154	14 JUNE 99	17 JUNE 98

TITLE OF INVENTION:

PHARMACEUTICAL FACTOR VII PREPARATION

APPLICANT(S) FOR DO/EO/US

MATTHIESSEN, Peter, TURECEK, Peter, and SCHWARZ, Hans-Peter

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S. C. 371 (c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☒ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (informal)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter. (informal)
16. ☐ Other items of information:

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
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17. ☒ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO ..... $		
International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... $		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $860.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provision of PCT Article 33(2)-(4) ..... $		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	27 - 20 =	7	x **$18.00**	**$126.00**	
Independent claims	4 - 3 =	1	x **$80.00**	**$80.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			**+$270.00**		
	TOTAL OF ABOVE CALCULATIONS =			**$1,066.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.0, 1.27, 1.28).					
SUBTOTAL =				**$1,066.00**	
Processing fee of **$130** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					
TOTAL NATIONAL FEE =				**$1,066.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40** per property +					
TOTAL FEES ENCLOSED =				**$1,066.00**	
				Amount to be refunded	$
				Charged	$

a. ☒ A check in the amount of $1,066.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayments to Deposit Account No. 16-2230. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted ton restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

OPPENHEIMER WOLFF & DONNELLY LLP
500 Newport Center Drive, Suite 700
Newport Beach, California 92660
Attn: Louis C. Cullman
Customer No: 25204

SIGNATURE:

Louis C. Cullman
NAME

39,645
REGISTRATION NUMBER

PATENT

Applicant: Matthiessen, et al.
Serial No.: Not Yet Assigned
Filed: December 15, 2000
Title: **PHARMACEUTICAL FACTOR VII PREPARATION**
Examiner: Unassigned
Group Art Unit: Unassigned
Atty Docket No.: 20560-27

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Preliminary to examination in this National Phase Application, please amend the above-identified application as outlined below.

IN THE SPECIFICATION PLEASE INSERT NEW TITLE

Page 1, please insert the following new title:

--STABLE BLOOD COAGULATION INHIBITOR-FREE FACTOR VII PREPRATION AND METHOD FOR PREPARING SAME--.

Page 1, before the very first paragraph, please insert the following heading:

--FIELD OF THE INVENTION--.

Page 1, before the second paragraph, please insert the following heading:

--BACKGROUND OF THE INVENTION--.

Page 3, line 10, delete the word "autokatalytic" and substitute therefor

--autocatalytic--.

Page 4, before the first full paragraph beginning "According to the invention...", please insert the following heading:

--SUMMARY OF THE INVENTION--

CERTIFICATE OF MAILING

I hereby certify that on December 15, 2000, this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service with sufficient postage in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, DC 20231.

37 C.F.R. § 1.8(a) ☐with sufficient postage as first class mail

37 C.F.R. § 1.10 ☒as "Express Mail Post Office to Addressee" Mailing Label No. EL591284467US

Patty Rasmussen

PATENT

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Page 1, before the second paragraph, please insert the following heading:

--BACKGROUND OF THE INVENTION--.

Page 3, line 10, delete the word "autokatalytic" and substitute therefor

--autocatalytic--.

Page 4, before the first full paragraph beginning "According to the invention...", please insert the following heading:

--SUMMARY OF THE INVENTION--

Page 4, at the end of the first full paragraph following the sentence ending in "blood coagulation." please insert --Stability or "stable" is, as known to those of ordinary skill in the blood coagulation arts, defined herein as a preparation containing blood coagulation Factor VII made in accordance with the teachings of the present invention that retains its biological activity after purification and upon standing. Biological activity is measured by activating the Factor VII and measuring its serine protease activity as described in Example 4 . A stable Factor VII preparation will retain a serine protease activity of at least 50 units/mg of protein. Units are defined consistent with the Immuno AG Immunochrom FVII:C package insert which is incorporated herein by reference in its entirety.--

Page 4, three lines from bottom, following the words "As soon as a"

insert --Factor VII--.

Page 4, two lines from the bottom, following the word "obtained" insert --in the Factor VII preparation--.

Page 5, before the first full paragraph at line 4, insert the following new paragraph:

--Blood coagulation Factor VII exists as a zymogan (an inactive form of enzyme) in the circulating blood. Factor VII is activated into Factor VIIa by Factor VII specific proteases such as Factors IXa and Xa. Once activated, Factor VIIa exerts a serine protease specific activity that mediates the activation of Factor X.--

Page 6, three lines from the bottom, delete the words "a lyophilisate" and substitute therefor –lyophilized--.

Page 8, before the first full paragraph beginning "As starting material for producing…" please insert the following heading:

--DETAILED DESCRIPTION OF THE INVENTION--

Page 19, eight lines from the bottom, delete the word –amidolytic" and substitute therefor --serine protease--.

Page 21, after the last paragraph, please add the following paragraph

--The present invention has been described in detail herein and with reference to cited publications. The publications cited are intended to provide the reader with additional information, not deemed essential to patentability. However, all cited publications are herein incorporated by reference in their entirety.--

IN THE ABSTRACT

Please delete the entire Abstract and substitute therefor:

--Stable pharmaceutical preparations containing blood coagulation Factor VII is disclosed. The pharmaceutical preparations containing blood coagulation Factor VII are free of coagulation inhibitors and are stable over a wide range of environmental conditions. Also provided are blood coagulation Factor VII preparations having a minimum activity of 50 Units/mg of protein that contain less than 5% activated blood coagulation Factor VII (Factor VIIa). The blood coagulation Factor VII containing preparations may also contain other blood coagulation factors and are free from detectable transmissible human pathogens.--

IN THE CLAIMS

Please cancel Claims 1 through 19.

Please add new claims 20 through 46 as follows:

--20. A stable blood coagulation factor preparation comprising:

blood coagulation factor VII having a specific protease activity, when activated, of at least 50 Units(U)/mg of total protein wherein in said blood coagulation factor preparation is free from blood coagulation inhibitors and contains less than approximately 5% of activated blood coagulation factor VII (blood coagulation factor VIIa).

21. The stable blood coagulation factor preparation of claim 20 wherein said blood coagulation factor VII has a specific protease activity, when activated, of greater than 100 Units/mg of total protein.

22. The stable blood coagulation factor preparation of claim 20 wherein said blood coagulation factor VII is present in an amount of between approximately 5 U/mL to approximately 5,000 U/mL.

23. The stable blood coagulation factor preparation of claim 20 wherein said preparation is lyophilized.

24. The stable blood coagulation factor preparation of claim 23 wherein said preparation is stable for at least 12 hours after reconstitution.

25. The stable blood coagulation factor preparation of claim 20 wherein said blood coagulation factor VII is a recombinant protein.

26. The stable blood coagulation factor preparation of claim 20 wherein said blood coagulation factor VII is recovered from normal human plasma.

27. The stable blood coagulation factor preparation of claim 26 wherein said blood coagulation factor preparation has no detectable transmissible human pathogens.

28. A method for preparing a stable blood coagulation factor preparation comprising:

absorbing blood coagulation factor VII from a biological material onto a chromatographic substrate;

selectively eluding said absorbed blood coagulation factor VII from said chromatographic substrate using a blood coagulation inhibitor-free elution buffer; and

selecting an eluate having a specific protease activity of at least 50 U/mg of total protein.

29. The method for preparing a stable blood coagulation factor preparation of claim 28 wherein said elution buffer has a pH of between approximately 5.0 to approximately 9.0.

30. The method for preparing a stable blood coagulation factor preparation of claim 29 wherein said elution buffer has a pH of between approximately 6.0 to approximately 7.5.

31. The method for preparing a stable blood coagulation factor preparation of claim 31 wherein said chromatographic substrate is an anion exchange material and said selective elution being performed using a chromatography column and a chromatography column flow rate of at least 0.15 column volumes per minute.

32. The method for preparing a stable blood coagulation factor preparation of claim 31 wherein said flow rate is between approximately 0.17 to 2.0 column volumes per minute.

33. The method for preparing a stable blood coagulation factor preparation of claim 28 wherein said chromatographic substrate is an immunoaffinity column specific for factor VII.

34. The method for preparing a stable blood coagulation factor preparation of claim 28 wherein said chromatographic substrate is a material having hydrophobic groups.

35. The method for preparing a stable blood coagulation factor preparation of claim 28 wherein said chromatographic substrate is a hydrogel.

36. The method for preparing a stable blood coagulation factor preparation of claim 28 wherein said biological material is selected from the group consisting of blood, plasma, a plasma fraction, a cell culture and a cell culture fraction.

37. The method for preparing a stable blood coagulation factor preparation of claim 31 further comprising absorbing said eluate having a specific protease activity of at least 50 U/mg of total protein onto a second chromatographic substrate having hydrophobic groups and selectively eluding said absorbed eluate from said chromatographic substrate having hydrophobic groups.

38. A pharmaceutical preparation made according to claim 28.

39. A pharmaceutical preparation made according to claim 37.

40. A stable blood coagulation factor preparation comprising:

blood coagulation factor VII having a specific protease activity, when activated, of at least 50 Units(U)/mg of total protein wherein in said blood coagulation factor preparation is

free from blood coagulation inhibitors and contains less than approximately 5% of activated blood coagulation factor VII (blood coagulation factor VIIa);

at least one additional coagulation factor.

41. The stable blood coagulation factor preparation of claim 40 wherein said blood coagulation factor is selected from the group consisting of factor II, factor IX and factor X.

42. A method for preparing a stable blood coagulation factor preparation comprising:

absorbing blood coagulation factor VII from a biological material onto an anionic chromatographic column;

selectively eluding said absorbed blood coagulation factor VII from said chromatographic column at a flow rate of between approximately 0.17 to 2.0 column volumes per minute using a blood coagulation inhibitor-free elution buffer having a pH of between approximately 6.0 to 7.5; and

selecting an eluate having a specific protease activity of at least 50 U/mg of total protein.

43. The method for preparing a stable blood coagulation factor preparation of claim 42 wherein said biological material is selected from the group consisting of blood, plasma, a plasma fraction, a cell culture and a cell culture fraction.

44. The method for preparing a stable blood coagulation factor preparation of claim 42 further comprising absorbing said eluate having a specific protease activity of at least 50 U/mg of total protein onto a second chromatographic substrate having hydrophobic groups and selectively eluding said absorbed eluate from said chromatographic substrate having hydrophobic groups.

45. A pharmaceutical preparation made according to claim 42.

46. A pharmaceutical preparation made according to claim 44.--

REMARKS

If it is felt for any reason that direct communication with Applicant's attorney would serve to advance prosecution of this case to finality, the Examiner is invited to call the undersigned attorney at the below listed telephone number.

The Commissioner is authorized to charge any fee which may be required in connection with this Amendment to deposit account No. 16-2230.

Respectfully submitted,

Dated: December 15, 2000

Louis C. Cullman
Registration No. 39,645

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Pharmaceutical Factor VII Preparation

The invention relates to a pharmaceutical preparation based on blood coagulation factor VII as well as to a factor VII purification method.

The coagulation of blood is triggered by a series of successive reactions of various blood coagulation factors. A deficiency of blood coagulation factors prevents the formation of fibrin from fibrinogen and thus prevents wound closure; the consequence is an increased bleeding risk or hemorrhages, respectively. This is the case if there is a deficiency of vitamin K-dependent blood coagulation factors, such as factors II, VII, IX and X, which is mainly caused by an impaired function of the liver but may also be caused by an inherited deficiency of blood coagulation factors. For a substitution treatment, the corresponding blood coagulation factors are employed. In most instances, a treatment with these preparations leads to a rapid hemostasis.

Factor VII may be recovered from a biological material, such as blood, plasma or cell cultures. If blood or plasma are the starting material, it is mostly obtained together with at least one of the structurally similar factors II, IX or X in purified form. A prothrombin-complex preparation based on factors II, VII, IX and X, or a plasma fraction containing the prothrombin complex, respectively, may likewise be used

as the starting material for preparing a further purified factor VII preparation.

For the treatment of patients who suffer from a factor VIII deficiency and who have developed an inhibitor directed against factor VIII, a factor VIIa preparation frequently has been suggested. Highly purified factor VIIa preparations have been described e.g. in EP 0 082 182 and by Hedner et al., (Haemostasis 19, 335-343 (1989)).

Factor VII is relatively easily activatable to factor VIIa. For instance, it has been found that factor VII zymogen is rapidly activated by a number of physiological enzymes, such as factor IXa and factor Xa (Wildgoose et al., Blood, Vol. 73, No. 7, 1989, pp. 1888-1895).

In EP 0 770 625 it has been described that with increasing complexity of the purification procedure, activation of factor VII occurs. Accordingly, the addition of blood coagulation inhibitors, such as antithrombin III/heparin or reversible inhibitors, such as benzamidine, during an affinity-chromatographic purification have been suggested to guard against the risk of a factor VIIa formation.

Likewise, the use of benzamidine protects the factor VII molecule from proteolysis during its isolation throughout the purification procedure, as described by Radcliffe et al., Journal Biological Chemistry 250,

1975, pp. 388-395.

The problem of a factor VII activation, primarily in the presence of positively charged surfaces, e.g. in case of contact with an anion exchanger material, has been described by Pedersen et al. (Biochemistry, 28, 1989, 9331-9336). It has been found that recombinant factor VII could be purified to a homogenous protein in the presence of benzamidine. In the absence of the inhibitor, recombinant factor VII was activated spontaneously. This autokatalytic activation therefore also is a problem in preparations in which not even traces of the physiological activation components are present any longer.

Inhibitors of blood coagulation as such are not desired in a pharmaceutical preparation for treating conditions caused by the deficiency of a blood coagulation factor. Physiological inhibitors, such as antithrombin III or heparin, are being added for stability purposes, i.e. to avoid the activation of the blood coagulation factors in prothrombin complex preparations. Yet, it would be desirable to provide preparations which are sufficiently stable as far as possible without the addition of such inhibitors.

The object of the invention is to provide a pharmaceutical factor VII preparation comprising a portion of activated factor VII as low as possible and a sufficient stability in the absence of inhibitors of blood

coagulation. Moreover, a purification method for producing factor VII preparations is to be provided which can be performed efficiently and which is gentle on the proteins, so as to avoid the use of inhibitors, such as benzamidine.

According to the invention, this object is achieved by a preparation based on blood coagulation factor VII comprising a portion of factor VIIa of less than 5%, having a specific activity of at least 50 U/mg and having a stability in the absence of inhibitors of blood coagulation.

According to the invention, it is possible for the first time to provide a highly purified factor VII preparation without using the known inhibitors of blood coagulation, in particular without the addition of antithrombin III and/or heparin, benzamidine, soybean trypsin inhibitor, phenyl-methyl-sulfonyl fluoride or EDTA. It has been shown that under the conditions described below, factor VII is not activated during a chromatographic purification procedure, even without the protection against proteolytic enzymes or activation by contact. Thus, the highly purified factor VII preparation does not necessarily contain any one of the inhibitors listed, or less than the detection limit thereof, respectively. As soon as a specific activity of at least 50 U/mg has been obtained, surprisingly it could be shown that factor VII is extremely stable

(even with regard to autocatalytic activation processes) whithout requiring the addition of a specific FVII activation inhibitor.

The stability of the highly purified factor VII is mainly - though not exclusively (cf. Pedersen et al. 1989, regarding an autocatalytic activation) - due to the depletion of factor VII-specific proteases, among them factors IXa and Xa. Thus, it is ensured that a preparation, such as a pharmaceutical infusion preparation, based on highly purified factor VII comprising at least 50 U/mg of protein, preferably at least 100 U/mg, most preferred at least 500 U/mg of protein, in special cases even at least 1000 U/mg up to the theoretical purity of approximately 2000 U/mg, can remain in a state ready for use over an extended period of time even in the absence of inhibitors of blood coagulation, without the portion of factor VIIa in the preparation increasing beyond the allowable extent.

The preparation according to the invention has a portion of less than 5% of factor VIIa, based on the total amount of factor VII, preferably less than 3%, most preferred less than the detection limit (e.g. in the test according to Seligson et al., Haemostasis 13, 186-191, 1983).

The preparation according to the invention is, e.g., a pharmaceutical grade concentrate which can be used to produce pharmaceutical combination prepara-

tions. Such combination preparations may comprise further active substances, such as vitamin K-dependent proteins, among them one or more of blood factors II, IX, X, protein C or protein S. Thus, e.g., a prothrombin complex preparation comprising factors II, VII, IX and X may be made into a partial prothrombin complex by admixing the factor VII preparation of the invention.

Due to its negligible load of contaminants, the factor VII infusion preparation according to the invention may be provided in relatively highly concentrated form, e.g. with a concentration of from 50 to 5000 U/ml. This substantially simplifies the administration of the preparation as a bolus injection or as a short-term infusion.

The stability of the preparation may be tested in the ready for use state so as to determine whether or not it meets the stability criteria according to the invention, i.e. by incubation at room temperature for a period of at least 12 h, preferably more than 30 h. In doing so it can be determined that the preparation according to the invention still comprises less than 5% of factor VIIa.

The preparation according to the invention may also be provided in a durable commercial form, preferably as a lyophilisate. Upon reconstitution, the extreme stability is shown again, and even during lyophilisation/reconstitution no negative effects occur as re-

gards the (premature) FVII activation. Further forms are deep-frozen preparations or liquid preparations which are stable for an extended storage period, optionally after the addition of stabilizers, such as carrier proteins and/or carbohydrates, preferably at 4°C.

In any case, despite its stability properties (also as regards an autoactivation), factor VII in the preparation according to the invention is an activatable factor VII which can be activated, e.g. *in vivo*, without any problems and subsequently will be blood-coagulation-active according to native factor VII. Preferably, a native factor VII protein is employed, e.g. human plasmatic factor VII, or human recombinant factor VII. In the recombination of nucleic acids, also factor VII analogs may be used which, in any case, are activatable to an equal or even a higher extent (Sridhara et al., Am. J. Hematology 53, 66-71, 1996).

According to a further aspect, the present invention relates to a method of purifying factor VII from a biological material and producing the factor VII preparation according to the invention by adsorption of factor VII on a chromatographic material, fractionated elution of factor VII with a specific activity of at least 50 U/mg, whereby the elution is carried out with a buffer without the addition of inhibitors of blood

coagulation, and recovery of factor VII from the eluate.

As starting material for producing the factor VII preparation according to the invention, usually a complex, biological material is used. This includes blood, plasma, plasma fractions, cell cultures, or cell culture fractions, respectively. Yet also a pharmaceutical grade preparation may be used as the starting material which, in addition to factor VII, also comprises further proteins, e.g. a prothrombin complex preparation comprising factors II, VII, IX and X.

To avoid the risk of a transmission of human pathogenic infectious agents, among them viruses transmittable by blood, such as HIV and hepatitis viruses, e.g. HAV, HBV, HCV, HGV, and parvoviruses, yet also the infectious agents of BSE and CJD, a series of measures are taken. Factor VII may be subjected to a method for inactivating, or depleting, respectively, human pathogens each prior to or following chromatographic purification. Preferably, at least two measures are provided which effect the inactivation, or depletion, respectively, due to a different mechanism. Among them are chemical, physico-chemical and physical methods. The methods using virucidal substances preferably are employed prior to or during the chromatographic purification procedure so that the virucidal agent can be removed simultaneously with the purification of factor

VII.

Effective measures for inactivating viruses include, e.g., treatment with organic solvents and/or detergents (EP 0 131 740, EP 0 050 061, PCT/AT98/00090), treatment with chaotropic agents (WO 90/15613), heat treatment methods, preferably in lyophilized, dry or humid state (cf. EP 0 159 311), combination methods (EP 0 519 901) and physical methods. The latter cause the inactivation of viruses e.g. by irradiation with light, such as in the presence of photosensitizers (EP 0 471 794 and WO 97/37686).

Human pathogen depletion methods particularly include filtrations using ultrafilters, depth filters or nanofilters (cf. WO 97/40861, AT A 1029/97). Yet, also precipitation steps or other protein purification measures, such as adsorption, contribute to the depletion of possibly present pathogens.

The method according to the invention for purifying factor VII and producing a factor VII preparation comprises at least one chromatographic step. In doing so, factor VII is adsorbed and selectively eluted and fractions are recovered. For the further recovery of factor VII from the eluate, those fractions are chosen in which the specific activity is at least 50 U/mg of protein, preferably at least 100 U/mg.

As elution buffer, preferably a buffer which has a pH in the neutral range, such as in the range of 5-9,

preferably 6-7.5, and which has an ionic strength corresponding to a content of NaCl of less than 1 M is employed. As has previously been described, none of the inhibitors of blood coagulation listed are added to the elution buffer. Possibly present physiological inhibitors which exist in the starting material are separated already during adsorption and, optionally, in the subsequent purification of the adsorbed factor VII with a washing buffer so that in any case factor VII is recovered without an inhibitor content. Here, too, the extraordinary stability of the highly purified factor VII is shown which then may be subjected to the further preparation procedures common for producing a pharmaceutical or diagnostic preparation.

Chromatography is performed either in the batch method or in a column. For an improved control of the flow rate or contact period of factor VII with the chromatographic material, the column method is preferred. Preferred materials are carriers with positively charged ligands which may be employed as anion exchangers.

As the anionic exchangers, in principle all the anion exchangers based on carbohydrates or synthetic polymers may be employed which have an affinity to factor VII (prothrombin), such as, e.g., DEAE-Sephacel®, DEAE-Sephadex®, DEAE-Sepharose CL6B®, DEAE-Sepharose

Fast Flow®, QAE-Sephadex®, Q-Sepharose Fast Flow®, Q-Sepharose High Performance®, Q-Sepharose Big Beads® (all from Pharmacia);

DEAE-Tris-Acryl®, DEAE-Spherodex®, Q-Hyper-D® (all from Sepracor);

Macroprep DEAE®, Macroprep Q® (all from BioRad);

DEAE-Toyopearl®, QAE-Toyopearl®, Toyopearl Super-Q® (all from Tosohaas);

Protein PAK DEAE® (Waters);

Fractogel EMD-TMAE®, Fractogel EMD-DEAE®, Fractogel EMD-DMAE®, Licrospher 1000 TMAE®, Licrospher 1000 DEAE® and Licrospher 4000 DMAE® (all from MERCK).

In particular, pressure-stable ion exchangers are used, such as, e.g., Fractogel TMAE-EMD, Express IonQ, Sonree 30Q. Surprisingly it has been shown that even on these materials the phenomenon of activation of factor VII does not occur as soon as the period of contact with the anion exchanger material, or the retention time on the column, respectively, is kept short, e.g. less than 5 min. Accordingly, in a column preferably a flow rate of at least 2.5 cm/min, preferably 3.0 cm/min, is chosen for the elution of the adsorbed factor VII.

In most instances, the flow rate corresponds to at least 0.15 column volumes per minute, preferably 0.17,

most preferred 0.2 column volumes per minute.

Further materials for chromatographic purification are carriers with ligands which have a specific affinity to factor VII, such as tissue factor, antibodies and peptides. Further preferred materials comprise hydrophobic groups.

As the gel for the hydrophobic interaction chromatography, preferably Phenyl-Sepharose High Performance® (from Pharmacia), yet also other chromatographic gels, such as, e.g., Butyl-Sepharose®, Octyl-Sepharose®, Phenyl-Sepharose®, Phenyl-Sepharose Fast Flow High Sub®, Phenyl Sepharose Fast Flow Low Sub® (all from Pharmacia);
Fractogel TSK-Butyl® (from MERCK);
Macroprep-Methyl-HIC-Support®, Macroprep t-Butyl-HIC-Support® (all from BioRad);
TSK-Gel Butyl Toyopearl®, TSK-Gel Phenyl Toyopearl® and TSK-Gel Ether Toyopearl® (all from Tosohaas) are employed.

As the further carrier materials, also common gel filtration media, such as, e.g., Superose 12, Superdex 75, may be used.

According to a particularly preferred embodiment, also combinations of the chromatographic methods listed are used for producing a factor VII preparation, e.g.

the combination of the anion exchange chromatography and hydrophobic interaction chromatography. Optionally, this may be followed by a gel filtration for further purification. Accordingly, an anion exchanger preferably is used as the chromatographic material in the method according to the invention, and a material suitable for hydrophobic chromatography.

The factor VII purified according to the invention may not only be formulated to a pharmaceutical factor VII preparation by the common measures of dialysis/diafiltration, sterile filtration and concentration. Likewise, combination preparations may be provided which contain the factor VII purified according to the invention in addition to other active substances.

In particular, a prothrombin complex preparation may be provided according to the invention which contains at least one of the blood coagulation factors II, IX and X in addition to the highly purified and stable factor VII. These further blood coagulation factors preferably also are purified as individual factors before the combination preparation is provided by appropriate formulation. Such a preparation may be provided with or without inhibitors of blood coagulation. In particular, a heparin content may be provided, according to a recommendation by Menache et al., Thrombosis Diathes. Haemorrh. 33, 645-647 (1975) for producing

factor IX-containing pharmaceutical preparations.

The present invention thus also relates to a pharmaceutical preparation comprising a factor VII according to the invention, or a factor VII preparation according to the invention, respectively. This preparation preferably may contain at least one, in particular all, of the blood coagulation factors II, IX and X.

According to a preferred embodiment, the preparation according to the invention is formulated as a pharmaceutical infusion preparation.

Further additions to the preparation according to the invention are preferably antithrombin III and/or Atheplex which is an antithrombin III/heparin complex, prepared, e.g., according to EP 0 129 534.

The invention will be described in more detail by the following examples.

E x a m p l e 1 : Purification of factor VII from cryosupernatant by anion exchange chromatography on Fractogel TMAE-EMD

340 l of cryosupernatant are adsorbed on $Al(OH)_3$ and eluted with 22.5 g of Na_2HPO_4 x 2 H_2O/l (pH 8.5) comprising 1% (v/v) Tween 80 (from plants) and admixed with AT III/heparin complex (350 IU of heparin/kg of eluate, 40 IU of AT III/kg of eluate). The Tween-containing eluate is concentrated approximately 15-fold by ultrafiltration on a membrane having an exclusion limit of ≤30 kD and diafiltered against 10 volumes of 20 mM

Tris/HCl (pH 7.0) (Tris buffer). After 0.2 µ filtration and adjustment of the Tween concentration to 15% (v/v), it is incubated for virus inactivation for 3 h at 40°C. The solution diluted with Tris buffer to twice of its volume (3 l) is applied to a BPG100/165 Fractogel TMAE-EMD 650 M-column (from MERCK), subsequently washed with Tris buffer and thereafter washed with Tris buffer at increasing NaCl step gradients (50, 100, 150, 200, 250, 1000 mM/l), eluted and regenerated. The flow rate was at least 2.5 to 3 cm/min at a bed height of 16.5 cm.

Upon the addition of 43.8 IU of heparin/kg and 5.0 IU of ATIII/kg, the 200 mM NaCl eluate (approximately 5 l) was concentrated 60-fold to a protein concentration of 5 mg/ml by ultrafiltration on a membrane having an exclusion limit ≤30 kD. After diafiltration against a solution of 4.8 mM Na_3-citrate x 2 H_2O and 61.6 mM NaCl/l, the pH is adjusted to a value of 8.0 ± 0.5. The solution is frozen and lyophilized. The lyophilisate is moisturized up to a residual moisture of 7-8% and heated for virus inactivation for 10 h at 60°C and for 1 h at 80°C.

T a b l e 1

Result

Fraction	*Specif. Act.* [U FVII/mg prot.]*	*FVII-Activation** [U FVIIa/U FVII]*	*Purification Factor*
Plasma	0.02	-	1
$Al(OH)_3$ Tween eluate	5	0.05	200
TMAE-eluate	100	0.25	5,000
heat-treated lyophilisate	100	0.35	5,000

*: maximum theoretical specific activity: 2000 U of FVII/mg of protein

**: complete activation at a FVIIa/FVII ratio of 15-20

E x a m p l e 2 : Purification of factor VII from cryosupernatant by anion exchange chromatography on Fractogel TMAE-EMD and subsequent hydrophobic chromatography on phenyl-Sepharose

It is proceded as in Example 1 as far as to the production of the heat-treated preparation (bulk powder). The bulk powder is dissolved at its original volume with Milli Q-water (from Millipore) (protein concentration approximately 5 mg/ml), its salt content is increased from 60 to 2000 mM NaCl/l and it is applied to an XK50/96 Phenyl-Sepharose-HP column (from Pharmacia) which had been equilibrated in 20 mM Tris/HCl (pH 7.4; 2000 mM NaCl/l). Upon application of

75 ml of FVII bulk powder solution at a flow rate of 10 ml/min, it is subsequently washed with approximately 10 SV of equilibrating buffer, and thereafter it is washed, eluted, and regenerated, respectively, with the following NaCl steps:

1200 mM NaCl/l

850 mM NaCl/l

500 mM NaCl/l

0 mM NaCl/l, each in 20 mM Tris/HCl (pH 7.4)

Approximately 2.8 l of the 850 mM NaCl eluate are subsequently concentrated 5-fold by ultrafiltration on a membrane having an exclusion limit ≤30 kD and diafiltered against 20 mM ammonium hydrogencarbonate and lyophilized with sublimation of the salts. The salt-free lyophilisate was taken up in 1/50 of the original volume in 0.4% Na_3-citrate x 2 H_2O, 0.8% NaCl (pH 7.0), and re-buffered with slight purification at a flow rate of 2.5 ml/min on a XK26/100 Superose 12 column (from Pharmacia) equilibrated in the same buffer.

Table 2

Result

Fraction	*Specif. Act. [U FVII/mg prot.]*	*FVII-Activation [U FVIIa/U FVII]*	*FVIII-Yield [%]*	*Purification Factor*
Plasma	0.02	-	-	1
$Al(OH)_3$ Tween eluate	5	0.05	100	200
TMAE-eluate	100	0.25	80	5,000
heat-treated lyophilisate	100	0.35	61	5,000
Phenyl-Seph. eluate	500	0.4	43	25,000
Superose 12-eluate	1,000	0.5	35	50,000

Example 3 : Influence of the flow rate on the activation of factor VII on Fractogel TMAE-EMD

The purification of FVII from an $Al(OH)_3$ eluate, after virus inactivation with 15% Tween (application 36 mg of protein/ml of gel), was tested on an XK26/16.5 Fractogel TMAE-EMD 650 M column (from Merck) at different flow rates at 22°C. The conditions of chromatography correspond to those in Example 1.

T a b l e 3

Result

Flow rate [cm/min]	*Activation [U F7a/UF7]*
0.94	7.6
1.88	2.65
2.35	0.45
2.83	0.24

It has been shown that with an increasing flow rate, the content of activated factor VII decreases. Then, starting from a value of higher than approximately 2.5 to 3 cm/s, the activation rate will remain approximately constant.

E x a m p l e 4 : Test for determining the stability of the FVII preparation

4.1. Incubation conditions

100-300 µl aliquots of the FVII-containing eluates of the TMAE, and Phenyl-Sepharose-chromatography, respectively, prepared according to Examples 1 and 2, were incubated for 38 h at 22°C. After this period the amidolytic FVII activity (Immunochrom FVII:C, from IMMUNO AG), the FVIIa coagulation (Staclot VIIa-Rtf, from Diagnostica Stago), and the protein concentration (Bradford) were tested as compared to an aliquot immediately frozen at -20°C.

4.2 Activity tests

4.2.1. IMMUNOCHROM FVII:C

The factor VII activity was kinetically measured

under the conditions of a complete activation of FVII by thromboplastin and Ca^{2+} and the subsequent activation of likewise added FX with a chromogenic FXa substrate. It was proceded according to the manufacturer's recommendations.

4.2.2. STACLOT VIIa-rTF

With recombinant, soluble tissue factor, in the presence of phospholipid and FVII deficient plasma alone, the coagulation triggered by FVIIa can be measured with the assistance of a coagulometer. It was proceeded according to the manufacturer's, Diagnostica Stago's, instruction.

4.3 Results

Fraction	***Protein [mg/ml]***	***U FVII chrom/mg Spec. Act.***	***U FVIIa Activat. t=0***	***U FVIIa Activat. t=38***
TMAE, 200 mM	0.04-0.08	150-200	0.1-0.3	0.3-0.8
Phenyl-Seph., 750 mM	0.02-0.04	500-1000	0.1-0.2	0.1-0.3

It has been shown that with the preparations produced according to the invention, there is no activation of FVII worthy of mention, even after a storage for 38 hours at 22°C. This was the more surprising as in the factor VII preparations known so far, always a

substantial activation (i.a. also by autocatalysis) occurred which could only be avoided by the addition of specific inhibitors.

(new) C l a i m s :

1. A preparation based on blood coagulation factor VII having a portion of less than 5% of factor VIIa, characterized by a specific amidolytic activity of at least 50 U/mg and a stability in the absence of inhibitors of blood coagulation.

2. A preparation according to claim 1, characterized by a specific amidolytic activity of at least 100 U/mg.

3. A preparation according to claim 1 or 2, with a factor VII concentration of from 50 to 5,000 U/ml.

4. A preparation according to claim 1 or 2, in lyophilized form.

5. A preparation according to any one of claims 1 to 4, which in its ready-to-use state at room temperature is stable for a period of at least 12 h.

6. A preparation according to any one of claims 1 to 5, characterized in that factor VII is an activatable, recombinant factor VII.

7. A preparation according to any one of claims 1 to 5, characterized in that factor VII is a native, plasmatic factor VII.

8. A preparation according to any one of claims 1 to 7, obtainable by a chromatographic purification method and fractionated elution of factor VII without addition of inhibitors of blood coagulation.

9. A preparation according to any one of claims 1 to 8, characterized in that it is formulated as a pharmaceutical infusion preparation.

10. A method for purifying factor VII from a biological materials and producing a factor VII preparation by adsorption of factor VII on a chromatographic material, fractionated elution of factor VII with a specific amidolytic activity of at least 50 U/mg, the elution being performed with a buffer without addition of inhibitors of blood coagulation, and recovery of factor VII from the eluate.

11. A method according to claim 10, characterized in that an anion exchanger in a column is employed as the

AMENDED SHEET

chromatographic material and the flow rate of elution is at least 0.15 column volumes per minute.

12. A method according to claim 10, characterized in that a carrier with hydrophobic groups is employed as the chromatographic material.

13. A method according to claim 10, characterized in that a carrier suitable for gel filtration is employed as the chromatographic material.

14. A method according to any one of claims 10 to 13, characterized in that factor VII is purified from blood, plasma, a plasma fraction, a cell culture or a cell culture fraction.

15. A method according to any one of claims 10 to 14, characterized in that factor VII is recovered from the eluted fraction which contains factor VII with a specific activity of at least 100 U/mg.

16. A method according to any one of claims 10 to 15, characterized in that an anion exchanger is employed as the chromatographic material, and a material suitable

for hydrophobic chromatography is employed as further chromatographic material.

17. A pharmaceutical preparation comprising a factor VII obtainable according to the method according to any one of claims 10 to 16.

18. A preparation according to claim 17, further comprising at least one of blood coagulation factors II, IX and X.

19. A preparation according to claim 17 or 18, further comprising heparin, optionally in the presence of antithrombin III, or Atheplex, respectively.

Pharmaceutical Factor VII Preparation

A b s t r a c t :

A preparation based on blood coagulation factor VII comprising a portion of less than 5% of factor VIIa and exhibiting a specific activity of at least 50 U/mg and a stability in the absence of inhibitors of blood coagulation, as well as a method of producing the same are described.

Docket No. PATENT 20560-27

COMBINED DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

This declaration is of the following type:

☐	original	☐	divisional
☐	design	☐	continuation
☐	supplemental	☐	continuation-in-part
☒	national stage of PCT		

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled PHARMACEUTICAL FACTOR VII PREPARATION, the specification of which:

(a) ☐ is attached hereto

(b) ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

(c) ☒ was described and claimed in PCT International Application No. PCT/AT99/00154, filed on 14/June/1999 and as amended under PCT Article 19 on _____ (*if any*).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application of which priority is claimed.

(d) ☐ no such applications have been filed

(e) ☒ such application have been filed as follows:

Prior Foreign Application(s)

Country (or indicate if PCT)	Application Number	Date of Filing (day, month, year)	Priority Claims Under 37 USC 119
Austria	A 1043/98	17/June/1998	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a), regarding events which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status-patented, pending, abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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